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UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION (UCBT) WITHOUT SEROTHERAPY IN CHILDREN IS ASSOCIATED WITH RAPID CD4 + T-CELL RECONSTITUTION AND RECOVERY OF ANTI-VIRAL RESPONSES

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Conventional UCBT with serotherapy is characterized by a low incidence of GvHD but delayed immune reconstitution and high infection-related mortality. We evaluated early immune reconstitution, infection-related mortality and incidence of GvHD in children undergoing unrelated UCBT without serotherapy.

13 children with a median age of 12 months (range, 1-144) underwent an unrelated UCBT for SCID (7), high risk/relapsed ALL (4) and secondary AML (2). Conditioning was myeloablative in 11 patients, reduced intensity in 1 patient and none in 1. HLA matching was 6/6, 5/6 and 4/6 in six, six and four cases respectively (3 double cord infusions). GvHD prophylaxis was cyclosporine/mycophenolate mofetil (11), cyclosporine/methylprednisolone (1) and cyclosporine (1). The median number of nucleated, CD34+ and CD3+ cells infused was 8.8×10^7/kg (range, 4.7-17.72), 3.8×10^5/kg (range, 1.23-11.95) and 6.1×10^6/kg (range, 1.9-24.45), respectively.

Median time to myeloid and platelet engraftment was 21 (range, 13-35) and 39 (range, 32-71) days, respectively. One patient died from conditioning related toxicity. 12 patients are alive and in remission with a median follow up of 12 months (range, 3-60). 10 patients have full donor chimerism while 2 patients have full donor chimerism in the lymphoid compartment only.

The incidence of aGvHD was high (grade II n = 7; grade III-IV n = 3) but generally responded promptly to steroids. At last follow-up, 8 patients are off immunosuppression, and one developed limited cGvHD. There was no infection-related mortality and low incidence of viral reactivations (n = 3 adenoviraemia).

Our most striking finding was of rapid CD4 + T-cell reconstitution. The median CD3+, CD4+ and CD8+ counts within 2 months post UCBT was 0.83 x10^9cells/L (range, 0.44-2.44), 0.63 x10^9cells/L (range, 0.06-1.89), and 0.15 x10^9cells/L (range, 0.01-1.21) respectively. This contrasts to a similar group of children (7) who underwent unrelated UCBT with serotherapy in whom the median CD3+/CD4+/CD8+ counts at 2 months were 0.07/0.03/0.03 x10^9cells/L. In our cohort 5 of 9 evaluable patients had normal CD4+ counts by 5-6 months post transplant.

Adenovirus specific T cells were detected as early as 60 days post transplant, and all 3 cases of adenoviraemia resolved rapidly.

We conclude that unrelated UCBT without serotherapy, while associated with a higher incidence of steroid-responsive aGvHD, results in rapid CD4 + T-cell reconstitution and a low incidence of viral complications.

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ENHANCED IMMUNE RECONSTITUTION DESPITE IN VIVO LYMPHO-DEPLETION AS PART OF ACUTE GRAFT VS HOST DISEASE AGVHD PROPHYLAXIS IN UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTS UHSCT

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Background: Slow lymphocyte recovery and low immunoglobulin levels post UHSCT may contribute to worse clinical outcomes.(relapse and survival). We reported preliminary low rates of aGVHD, infections and treatment related toxicity with the use of Thymoglobulin (4.5 mg/kg divided doses on days -1,-2, & -3), Tacrolimus and Sirolimus for aGVHD prophylaxis in adult UHSCT. Here we explored the differences in absolute lymphocyte count ALC and Immunoglobulin IgG levels at three time points (days 30, 60, & 90) post transplant in relation to a large comparable group of historical patients at our center treated with Tacrolimus and Mycophenolate (TM) as aGVHD prophylaxis.

Methods: Analyses are made on non-censored and censored data (for aGVHD and relapse events) in TTS vs TM groups. The original measurements ALC & IgG are tested at each time point (30, 60, and 90 days) for group differences via the Student's t-test. The dichotomized measurements (thresholds of ALC at 1000/μl and IgG at 500/μl) are compared between two groups using Fisher's exact test. The difference in the linear trends of ALC and IgG thresholds between two treatment groups is investigated using logistic model and Wald Chi-square test for interaction of day and group.

Results: There were 25 patients on TTS and 72 on TM. The incidence of aGVHD was higher in TM 77.8% compared to 24% in TTS (P< 0.0001). No difference in the relapse rate (12.5% TM vs 16% TTS P 0.7354). We observed a higher mean IgG level in TTS vs TM groups at days 60 & 90 post transplant (P 0.0558 & 0.0087), In addition to higher percentage of patients with an IgG level > 500 mg/μl on days 30 & 90 (P 0.0358, 0.0078). When patients with aGVHD & relapse were excluded from both groups, there was no statistical deference between TTS & TM. Furthermore the percentage of patients with ALC > 1000/μl in TTS nearly tripled in a linear fashion from day 30 to day 90 (20 to 59% trend test P 0.0086 for non censored & 17% to 58% P 0.0081 for censored), while the same percentage nearly doubled for TM group between days 30 and 90(21 to 46% P 0.0025 for non censored & 26 to 63 P 0.0017 for censored).

Conclusion: These early results suggest that there is an enhanced immune reconstitution in the experimental group TTS despite lympho-depletion, as shown by higher IgG as well as a steep linear recovery of ALC. We postulate that the decreased aGVHD in TTS resulted in less need for additional immune suppression and hence improved immune reconstitution.

Comparing dichotomized biomarkers between Experimental and historical control groups on noncensored data

	Control		Experimental		
	Percent above threshold	p value	Percent above threshold	p value	p value
Immunoglobulins = >500					
Day 30	76%	0.3591*	96%	0.6334*	0.0358†
Day 60	78%		92%		0.2172†
Day 90	69%		100%		0.0078†
Lymphocytes = >1000					0.4332 ‡
Day 30	21%	0.0025*	20%	0.0086*	1.0000†
Day 60	47%		50%		0.3322†
Day 90	36%		59%		0.4164†

† P-values from two-sided Fisher's exact test for the difference between two groups at each time point. * P-values from the Cochran-Armitage Trend Test for the linear trend across 3 time points within each group ‡: P-values from the Wald Chi-square test for linear trend difference between two groups

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THE ABSENCE OF VASOACTIVE INTESTINAL PEPTIDE AUGMENTS ALLOREACTIVITY AND THE ANTI-VIRAL RESPONSE IN A BONE MARROW TRANSPLANT SETTING

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Backgrounds: Vasoactive intestinal peptide (VIP) can lead to the generation of regulatory dendritic cells (DC). In this study, we used DC and T-cells from VIP-knockout (VIP-KO) mice to investigate the effects of VIP on allogeneic and anti-viral immune responses in transplant recipients.

Methods: 5×10^6 BM and 1, 3, 8×10^6 splenocytes (SP) from VIP-KO and WT donors, single VIP-KO donors, single WT donors, or single VIP-heterozygous donors were transplanted in B6 → B10BR alloBMT and B6 → B6 synBMT models. Survival and GvHD were

assessed. VIP-KO mice, WT littermates, and recipients were infected with 5×10^4 PFU murine cytomegalovirus (mCMV) and T cell response to viral antigen was measured by flow cytometry for mCMV peptide-MHC class I-tetramer$^+$ CD8$^+$ T-cells at day 0, 3, 7, 10, and day 15 post infection or 80, 83, 87 and 101 days post-transplant (infection at day 80 post-transplant). Day 15 post mCMV challenge, VIP-KO and WT mice were euthanized. DC and T-cells were purified from BM and SP by FACS and MACS, respectively. 2×10^5/mL DC treated with 1×10^4 PFU mCMV peptide-expressing Listeria-CMV construct and incubated with 2×10^6/mL T-cells at 37 °C. Cultured 3 days and 7 days, cells were harvested and analyzed with DC and T-cell surface marker, tetramer, and intracellular cytokines by flow cytometry.

Results: allogeneic recipients of VIP-KO BM and VIP-KO SP developed more GvHD than recipients of WT grafts using a lower dose of donor SP (1×10^6), while there was no difference in survival. The GvHD scores and the percentage of survival showed no difference among other syngeneic or allogeneic BMT settings.The specific anti-viral immunity was similar among the non-transplanted VIP-KO mice, and allogeneic and syngeneic transplant recipients of VIP-KO donor cells. 3 and 7 days post culture, VIP-KO DC expressed higher-level of CD80, MHC-II and lower-level of PD-L1, VIP-KO T-cells had higher-level of tetramer$^+$ CD8$^+$ T-cells and intracellular IFN-γ, lower-level of IL-4 and IL-5, PD1, and ICOS. Taken together, these observations suggest that VIP expressed on immune cells suppresses anti-viral immune responses and Th1 polarization.

Conclusion: The anti-viral immune responses of VIP-KO immune cells were independent allogeneic immunity; VIP expressed by neurocrine cells in WT recipients did not compensate for the lack of VIP in mice transplanted with VIP-KO cells. Modulation of the VIP pathway is a novel method to regulate post-transplant immunity allogeneic transplant recipients.

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THE LIVER MAINTAINS STRONG POPULATIONS OF INNATE IMMUNE CELLS THAT CONTRIBUTE TO HOST PROTECTION AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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The liver is a critical immunocompetent organ armed with lymphocytes, natural killer (NK) cells, and a variety of antigen-presenting cells (APC), including resident macrophages, called Kupffer cells (KC). Exposed to large amounts of both destructive and harmless toxins and antigens, the liver must provide immunogenic and tolerogenic immunity. Here, we studied the role of the liver after hematopoietic cell transplantation (HCT). Lethally irradiated BALB.K mice received MHC-matched, purified hematopoietic stem cells (HSC; cKit + Sca1 + Thy1.1loLin-) +/- splenocytes (SP) from AKR/J donors. Ficoll-separated mononuclear cells (MNC) from PBS flushed livers were FACS analyzed post-HCT (pTX). In recipients of HSC + SP the liver was a major target organ of acute graft-vs-host disease (GVHD) with prominent donor T cell (TC) expansion, while NK cell (DX5 + CD122+) and KC (CD11b + F4/80+) levels were severely decreased. HSC-derived donor cells were rare. In contrast, mice given pure HSC showed no signs of GVHD, and early pTX high proportions of NK cells and KC were present within the livers. NK cells comprised up to ~30% of cells and were mixed donor/host type, while KC were donor derived at 6w pTX. We hypothesized that rapid regeneration of KC may shield against the pathogen and toxin load entering the circulation from irradiation-damaged intestines. In fact, when KC reconstitution was suppressed by silica administration mice displayed severe weight loss, hunched posture, ruffled fur, diarrhea, and a >50% mortality. Survivors stabilized ~d12, presumably with gut recovery. To test if regenerating APC could protect against GVHD, a lethal dose of SP was given at 0, 4, 7, or 10d pTX, time points at which control livers contained 0, 11, 25, 32% KC, respectively. All mice receiving SP on d0 died, but death occurred in only 50%, 17% and 0% of mice when SP were given on d4, 7, and 10, respectively. Although donor chimerism decreased with delayed SP injection, lymphocyte reconstitution was improved. In conclusion, the role of the liver as an immunologically active organ after 'conventional' HCT is often masked by donor TC expansion and GVHD. Rapid recovery of innate liver immunity may protect the host from endotoxemia and mediate tolerance between donor and host. Elevated proportions of NK cells, which are lacking in GVHD affected mice suggest another beneficial mechanism. Immunohistochemical studies for a better quantitative assessment of resident liver immune cells are underway.

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A TREND FOR BETTER IMMUNE RECONSTITUTION AND LOWER INCIDENCE OF INFECTIONS AFTER UNRELATED CORD BLOOD TRANSPLANTATION IN CHILDREN COMPARED TO ADULTS

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In umbilical cord blood transplantation (UCBT), the lower infused cell dose might lead to an increased infectious risk. To get further insights on this issue, we retrospectively analyzed infectious events and immune restoration in 35 consecutive patients (pts) treated by UCBT from Jan 2005 to Dec 2008 in the University Hospital of Besançon.

There were 7 children and 28 adults aged 6 to 62 y (median 35y). All pts suffered from malignant diseases. Nine pts were in CR1, 10 in CR2 and 16 in ≥CR3 or in PR. Nine pts were CMV$^+$ and 29 EBV$^+$. A myeloablative conditioning regimen with 12 GY TBI (16pts) or Busulfan (2 pts) + 120 mg/kg cyclophosphamide + 75 mg/m^2 fludarabine was given to 18 pts, one child received ALG instead of fludarabine. A fludarabine based reduced intensity conditioning (RIC) was given to 17 pts. There were 29 double and 6 single unit transplants. The median follow-up is 638 days (259-1449).

All pts engrafted except 3 after RIC. Fifteen pts died (43%), 10 of relapse, 4 of infection (1 ARDS,1 zygomycosis, 1 fusariosis and 1 HHV6 encephalitis) and 1 died of post-conditioning toxicity. There were 8 infectious events in 7 children, (mean 1.14/pt) with 1 death in the pt who received ALG and 86 infectious events in 28 adults (mean 3.07/pt) causing 3 deaths.

Viral infections occurred in 27 pts (77%) in majority before D100 (73%). BKV$^+$ hemorrhagic cystitis (HC) occurred in 13 pts, VZV (6), CMV (5), HHV6 (1), HSV (1), and RSV (1). We recorded 22 documented bacterial infections caused by 13 Gram negative and 9 Gram positive agents. Invasive fungal infections were diagnosed in 9 pts (26%) with 6 proven or probable aspergillosis, 2 fungal septicaemias and 1 zygomycosis. Fungal infections seemed more frequent in pts experiencing chronic GVHD (35% versus 21.4%, hazard ratio = 1.6, p = 0.91).

The immune reconstitution appeared quicker in children, the median CD4 T cell count at 3-6-12 months post UCBT was 224-1008-1333/mm^3 in children and 119-216-364 in adults. Median B cell count at 6 mo post UCBT was 868 in children versus 106 in adults. Among the 20 survivors at 1 year post transplant, the vaccine response was complete in 15 pts, incomplete in 2, non available in 3.

The lower incidence of infectious events in children might be due to a better immune reconstitution. The overall infectious mortality rate is relatively low (11%). The high prevalence of HC advocates for a prospective follow-up of BK virus and the high rate of Zoster warrants preventive strategies.

LATE EFFECTS/QUALITY OF LIFE

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A PROSPECTIVE STUDY OF IRON-OVERLOAD (IO) MANAGEMENT IN ALLOGENEIC HEMATOPOIETIC-CELL TRANSPLANT (ALLO HCT) SURVIVORS

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While transfusional IO occurs in 30-60% of allo HCT survivors, the treatment of post-HCT IO is not well described. We